INFLUENCE OF PRENATAL EXPOSURE TO SELECTED ORGANOHALOGANS ON INFANT SEXUAL AND NEUROLOGICAL DEVELOPMENT

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Introduction

In 2000 the Compare project was launched for "Comparison of Exposure-Effect Pathways to Improve the Assessment of Human Health Risks of Complex Environmental Mixtures of Organohalogans". Within this project the Groningen-Infant-Compare (GIC) cohort was founded, consisting of 90 mother-infant pairs. Objective was to investigate influence of prenatal exposure to selected organohalogans on infant development, especially sexual and neurological development. 8 neutral and 4 phenolic organohalogans were selected to be analysed in maternal serum taken in the 35th week of pregnancy to determine prenatal exposure. The following neutral organohalogans were ana1ysed: 1 polychlorinated biphenyl (PCB: CB-153), the daughter compound of dichloro-diphenyl-trichloroethane (DDT: 4,4'-DDE), 5 polybrominated diphenyl ethers (PBDEs: BDE-47, BDE-99, BDE-100, BDE-153, BDE-154), and hexabromocyclododecane (HBCDD). The analysed phenolic organohalogans were 3 hydroxylated PCBs (4OH-CB-107, 4OH-CB-146, 4OH-CB-187), and pentachlorophenol (PCP). The selection of these organohalogans was based on their historical identification (PCB, DDT and PCP) and their more recent production (PBDEs and HBCDD). We investigated if prenatal exposure to this combination of organohalogans influences sexual development (sex hormone levels, testes volume and penile length) and neurological development of infants up to 18 months of age.

Materials and methods

GIC cohort. The women participating in the GIC cohort were recruited by their midwives or gynaecologists. Informed consent was obtained before participation. 56 boys and 34 girls were born within the cohort.
Organohalogan analyses. CB-153, 4,4'-DDE, PCP, and the 3 hydroxy-PCBs were analysed in 90 maternal serum samples at the Institute for Environmental Studies, Vrije Universiteit, Amsterdam (*1*). 5 PBDEs and HBCDD were analysed in 69 maternal serum samples at the Department of Environmental Chemistry, Stockholm University, Sweden (*2*).
Lipid analyses. Lipid weight basis (1.9+1.3 (total cholesterol+triglycerides)) was calculated based on the correlation between cholesterol, triglycerid and phospholipid content in human serum (*3*). Total cholesterol was determined by the CHOD-PAP method (Roche/Hitachi). Triglycerides were determined by the Trig/GB methods (Roche/Hitachi).
Sexual development. Sex hormones were analysed in serum of infants at 3 months, since peak levels can be found at this age and decline shortly thereafter (*4*). Inhibin B (IB), sex hormone binding globulin (SHBG), testosteron (T), luteinising hormone (LH), follicle stimulating hormone (FSH), and estradiol (E) were determined at the Endocrine Laboratory, Department of Internal Medicine, Erasmus Medical Centre, Rotterdam, The Netherlands (*5*). Free testosteron (FT) and free estradiol (FE) were calculated as described before (*6*). In 48 boys and 26 girls enough blood was drawn to determine levels of one or more sex hormones.
Testes volume was measured at 3 and 18 months of age, by 3 pediatric radiologists, trained for the examination. No significant difference in outcome between the radiologists was observed. With standard ultrasound machine height, length and width were determined 3 times and average of these 3 measurements was taken as outcome. Testes volume was measured in 46 boys at 3 months of age and 47 boys at 18 months of age. Penile length (from basis to tip of penis) was measured at 3 and 18 months of age, with a single standard tape line, by the same investigator. Penile length was determined in 51 boys at 3 months of age and 47 boys at 18 months of age.
Neurological development. Neurological development examinations were performed by the same investigator at the age of 10 days (Prechtl examination), 3 months (Touwen examination) and 18 months (Hempel examination and Bayley scales of infant development). The Prechtl examination consists of 7 analysed scores: optimality score, hypotonic score (decreased tone), dystonic score (aberrant tone), apathy score (hypo reflex, with low to absent spontaneous movements), hyper reflex score, asymmetric score (in posture and movement), and

visomotor score (position and movement of eyes). All 90 infants were examined. The Touwen examination consists of 6 analysed scores: optimality score, developmental score (according to or ahead of age), hyper excitability score (testing brainstem reflexes), apathy score, visomotor score, and hypotonic score. 89 infants were examined. The Hempel examination consists of 6 analysed scores: optimality score, apprehension score (mode and ability of grasping), posture score (according to age or delayed), balance (small or broad base), dystonic score, and variability score (in movements). 87 infants were examined. The Bayley scale of infant development-II consisting of 3 scales: mental, motorical, and behavioral scale. After completion a rough for mental and motor development score is calculated and the mental and motor development index is determined. The index has a mean value of 100, with a standard deviation of 15. 87 infants were examined.
Statistical analyses. Influence of prenatal exposure to organohalogans on sexual and neurological development was determined by multiple regression analyses with backward mode and an F-value of <0.05. Body weight and length are confounding factors for testes volume and penile length. Confounding factors for neurological examinations were: duration of breastfeeding, parity, APGAR score, gestation length, age mother, smoking and alcohol use during pregnancy, Obstetric Optimality Scale, Wechsler Adult Intelligence Scale, Home Observation for Measurement of the Environment. Statistical analyses were performed with SPSS 12.0.

Results
Maternal serum levels of organohalogans are presented in table 1. Except for BDE-99, HBCDD, and 4OH-CB-107, levels were above limit of detection and quantification in all samples analysed.
All sex hormone levels were above limit of detection except for T levels (in 8 out of 24 samples) and LH levels (in 11 out of 21 samples) in girls. Influence of prenatal exposure levels to organohalogans on sex hormone levels in boys and girls are presented in table 2. T and SHBG level in boys and FT and T level in girls were significantly influenced by prenatal exposure to several organohalogans. According to the model for T, an increase in CB-153 level with 17 ng/g lipid, from p50 (64 ng/g lipid) to p75 (81 ng/g lipid), without change in BDE-99 and BDE-154 level, will decrease T level with 0.4 nmol/L.
Prenatal exposure to several organohalogans significantly influenced testes volume at 18 months of age and penile length at 3 and 18 months of age (table 3). Increase in 4OH-CB-187 level with 1 pg/g fresh weight, without change in the other independent variables, will decrease testicular volume with 1.2 mm^3. Testes volume at 3 months of age was not influenced by prenatal exposure to one or more organohalogans.
Several neurological scores were significantly influenced by prenatal exposure to 1 or more organohalogans (table 4).
As can be observed in table 2, 3, and 4, prenatal exposure to organohalogans influences sexual and neurological development both in positive as well as negative manner, depending on the compound. The manner of influence of one single compound is consistent between the various outcome measures. As for instance for BDE-154, its prenatal exposure level positively influences T and SHBG level in boys, and also positively influences testes volume and penile length at 18 months of age.

Discussion
The sexual and neurological development of healthy infants up to 18 months of age is influenced by prenatal exposure to a number of organohalogans.
Influence of prenatal exposure to organohalogans on infant sexual development has not been published previously, especially not on testes volume and penile length. In wildlife there are indications of influence of prenatal exposure to organohalogans and sexual development aberrations. Guillette et al. extensively investigated alligator reproductive development. In these animals, they observed small penis size, poorly organized testes, and depressed testosteron level (*7-9*). Reproductive development anomalies have also been observed in Florida panthers (cryptorchidism), and male offspring of sewage sludge treated pregnant sheep (reduced testes weight, Sertoli cell, Leydig cell and gonocytes number) (*10,11*).
Influence of prenatal exposure to organohalogans on infant neurological development has been published previously. In 418 Dutch infants small negative effect was observed from prenatal exposure to PCBs on neurological condition in 18-months-old toddlers (*12*). To our knowledge we are one of the first to publish data on prenatal exposure to BFRs on infant neurological development.
Our study shows that background exposure to older and more recent introduced organohalogans (like brominated flame retardants) influence sexual and psychomotor development of newborn infants. Longer effects are not yet known.

Tables

Table 1. Levels (median and range) of neutral and phenolic organohalogans analysed in maternal serum, on lipid (ng/g lipid) and fresh weight basis (pg/g serum).

	Median	Range	n.d./n.q.		Median	Range	n.d./n.q.
	Lipid weight basis (ng/g lipid)				Fresh weight basis (pg/g serum)		
DDE	89	18-380		PCP	970	300-8500	
CB-153	63	19-230		4OH-CB-107	26	n.d.-120	3/-
BDE-47	0.8	0.04-6.1		4OH-CB-146	100	36-700	
BDE-99	0.2	n.d.-2.1	3/-	4OH-CB-187	80	36-480	
BDE-100	0.2	0.03-1.4		6OH-BDE-47	n.d.	n.d.	90/-
BDE-153	1.6	0.3-20					
BDE-154	0.5	0.1-3.5					
HBCD	0.7	n.d.-7.4	1/-				

n.d./n.q. = not detected/not quantified.

Table 2. Influence of prenatal exposure to organohalogans on sex hormone levels in boys and girls at 3 months of age.

Boys		Regression coëfficiënt	Girls		Regression coëfficiënt
T[a]	CB-153	-0.023*	**FT**[g]	BDE-47	0.001*
	BDE-99	-2.957**		BDE-100	-0.002**
	BDE-154	2.556**		HBCD	0.000*
SHBG[b]	BDE-99	-64.139**	**T**[h]	BDE-47	0.038*
	BDE-154	38.084*		BDE-100	-0.129*
LH[c]	HBCD	0.366**		HBCD	0.009*
FSH[d]	PCP	0.000**			
E[e]	BDE-154	13.216*			
IB[f]	BDE-154	67.052**			

[a] R^2: 0.402, intercept: 4.408, n: 32. [b] R^2: 0.261, intercept: 125.213, n: 32. [c] R^2: 0.317, intercept: 0.831, n: 33. [d] R^2: 0.237, intercept: 0.718, n: 41. [e] R^2: 0.243, intercept: 27.077, n: 29. [f] R^2: 0.297, intercept: 294.293, n: 36. [g] R^2: 0.556, intercept: 0.000, n: 21. [h] R^2: 0.451, intercept: 0.047, n: 21. * p<0.05, ** p<0.01.

Table 3. Influence of prenatal exposure to organohalogans and body weight and height on testes volume at 18 months of age, and penile length at 3 and 18 months of age.

		Regression coëfficiënt			Regression coëfficiënt			Regression coëfficiënt
Testes volume 18 months of age[a]	4OH-CB-187	-1.185*	**Penile length 3 months of age**[b]	PCP	+0.000**	**Penile length 18 months of age**[c]	CB-153	-0.020**
	BDE-47	-45.431*		4OH-CB-107	+0.026**		BDE-154	+0.417*
	BDE-154	+107.913**		4OH-CB-146	-0.010**		HBCD	+0.308**
	HBCD	+50.651**		Length	+0.129**		Length	+0.091*
	Length	+20.145**						

[a]: R^2: 0.552, intercept: -1240.084, N:36. [b]: R^2: 0.347, intercept: -3.679, N:51. [c]: R^2: 0.446, intercept: -1.893, N:36. * p<0.05, ** p<0.01.

Table 4. Influence of prenatal exposure to organohalogans on neurological outcome at 10 days, 3, and 18 months of age.

			Regression coëfficiënt				Regression coëfficiënt
Prechtl	Apathy score[a]	4OH-CB-107	+0.023*	**Bayley**	Mental classification[j]	4OH-CB-107	-0.010*
	Hyper reflex score[b]	4,4'-DDE	-0.007*			4OH-CB-146	+0.005**
	Asymmetric score[c]	4,4'-DDE	+0.004**			HBCD	-0.099*
		BDE-154	-0.283*		Mental development[k]	4,4'-DDE	+0.054*
	Visomotor score[d]	BDE-47	+0.385*			CB-153	+0.195**
		BDE-100	-1.960*			4OH-CB-146	-0.143**
Touwen	Optimality score[e]	4,4'-DDE	+0.024**		Motorical classification[l]	4OH-CB-107	-0.013*
	Developmental score[f]	4OH-CB-146	+0.013**			4OH-CB-187	+0.008*
		4OH-CB-187	-0.020*			BDE-47	-0.129*
Hempel	Posture score[g]	CB-153	-0.026**		Behavior score[m]	BDE-47	-3.286**
		BDE-47	+0.515*		Orientation score[n]	BDE-47	-1.750**
	Dystonic score[h]	4OH-CB-107	+0.021*		Emotion score[o]	4,4'-DDE	+0.018*
		4OH-CB-146	-0.008**			4OH-CB-187	-0.038*
	Variability score[i]	4,4'-DDE	+0.006*			BDE-100	-7.644**
		4OH-CB-107	-0.027*		Motorical quality score[p]	BDE-47	-1.518**
						BDE-99	+4.836**
						BDE-154	-1.357**
						HBCD	+0.341*

[a]R^2: 0.080, intercept: 1.354, N: 88. [b]R^2: 0.216, intercept: 2.838, N: 88. [c]R^2: 0.188, intercept: -0.165, N: 67. [d]R^2: 0.205, intercept: 0.156, N: 67. [e]R^2: 0.420, intercept: 7.386, N: 78. [f]R^2: 0.349, intercept: -3.425, N: 78. [g]R^2: 0.177, intercept: 5.680, N: 69. [h]R^2: 0.349, intercept: 5.786, N: 77. [i]R^2: 0.127, intercept: 2.221, N: 90. [j]R^2: 0.512, intercept: 5.918, N: 58. [k]R^2: 0.619, intercept: -293.607, N: 85. [l]R^2: 0.194, intercept: 2.114, N: 66. [m]R^2: 0.268, intercept: 116.455, N: 56. [n]R^2: 0.313, intercept: 44.174, N: 57. [o]R^2: 0.461, intercept: 68.160, N: 57. [p]R^2: 0.649, intercept: 18.840, N: 49. * $p<0.05$, ** $p<0.01$.

Acknowledgements
Financial support was given to the project "COMPARE" by the European commission RD (Life Science Program, QLK4-CT-2000-0261). We would like to thank all women and their infants who participated in this study; Ioannis Athanassiadis for extensive assistance with GC-MS analyses of the brominated compounds; and Frank Perton for determination of cholesterol and triglycerides.

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